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Neuropeptide Y, peptide YY and C-terminal fragments release histamine from rat peritoneal mast cells

[1]Lars Grundemar & Rolf Håkanson

Department of Pharmacology, University of Lund, Lund, Sweden

1 Neuropeptide Y (NPY) and peptide YY (PYY) seem to act on at least two receptor sybtypes, Y_1 and Y_2. The Y_1-receptor requires the whole C-terminally amidated NPY/PYY molecule whereas the Y_2-receptor in addition recognizes C-terminal fragments of the two peptides. The present study was designed to elucidate whether NPY and related peptides were able to release histamine from isolated peritoneal mast cells of the rat.

2 NPY, NPY 15-36, NPY 22-36, NPY 26-36 and desamido-NPY evoked a concentration-dependent release of mast-cell histamine. The pEC_{15} values for NPY 15-36 and NPY 22-36 were higher, while the pEC_{15} value for NPY 26-36 was lower than that for NPY. At the highest concentration tested (0.1 mM), NPY and its C-terminal fragments released between 30 and 40% of the total histamine content. At the same concentration desamido-NPY released about 20%.

3 PYY and PYY 15-36 also evoked a concentration-dependent release of mast-cell histamine. PYY was more effective than PYY 15-36 since, at 0.1 mM, PYY released about 33%, while PYY 15-36 released about 15% of the total histamine content. Pancreatic polypeptide (PP) and the Y_1-receptor-selective agonist [Pro^{34}]NPY were virtually inactive.

4 The effect profile of the NPY/PYY-related peptides suggests that they act on the mast cells by a mechanism that does not involve either of the receptor subtypes hitherto described. The kinetics of the NPY-evoked histamine release may suggest that positively charged amino acid residues of NPY/PYY release mast-cell histamine by a non-receptor mechanism, as has been suggested for substance P and other basic peptides.

Keywords: Neuropeptide Y; peptide YY; C-terminal fragments; histamine release; mast cells of rat

Introduction

Neuropeptide Y (NPY) is a 36 amino acid peptide with a wide distribution in the peripheral nervous system (Lundberg *et al.*, 1982b). It is found in many sympathetic fibres, notably around blood vessels (Ekblad *et al.*, 1984; Uddman *et al.*, 1985; Sundler *et al.*, 1986). Peptide YY (PYY) is a gut hormone (Lundberg *et al.*, 1982a; Böttcher *et al.*, 1984) that displays 70% homology with NPY. NPY and PYY seem to act on at least two receptor subtypes. The Y_1-receptor is located postjunctionally and is able to evoke vasoconstriction, whereas the Y_2-receptor seems to inhibit transmitter release (for a review see Wahlestedt *et al.*, 1990). We have recently shown that NPY elicits a biphasic blood pressure response (pressor response followed by a depressor response) in the anaesthetized rat, and that the depressor response is abolished by pretreatment with a histamine H_1-antagonist or with the histamine liberator compound 48/80, suggesting that the depressor response reflects release of mast cell histamine (Grundemar *et al.*, 1990). Similarly, recent data suggest that the depressor response evoked by various C-terminal NPY fragments is also abolished by pretreatment with histamine H_1-receptor antagonists (Wahlestedt *et al.*, 1990).

In the present study we examined whether NPY, PYY or C-terminal fragments of NPY and PYY are able to release histamine from rat peritoneal mast cells.

Methods

Adult male Sprague-Dawley rats (freely fed, body weight 300–350 g) were killed by decapitation. Peritoneal cells, consisting of 3–6% mast cells (Padawer, 1963; Kurose & Saeki, 1981), were obtained by peritoneal lavage with 10 ml buffered salt solution (BSS), containing NaCl 145 mM, KCl 2.7 mM and 10% (v/v) Sörensen phosphate buffer ($Na_2HPO_4 + KH_2PO_4$, 67 mM, pH 7.0). After gentle abdominal massage for 5 min, the intra-abdominal mast cell-rich fluid was removed with a pipette. The abdominal fluid was centrifuged at approximately 300 *g* for 5 min at room temperature. The precipitated cells were resuspended in 10 ml BSS to which was added 1 mg ml^{-1} bovine serum albumin (BSA) (Armour, Kankakee, IL, U.S.A.). The cell suspension was washed twice with BSA. Finally, the cells were suspended in 20 ml BSA at room temperature and aliquots of 90 μl were transferred to polypropylene test tubes and preincubated for 5 min at 37°C before adding the peptide in a volume of 10 μl BSS. Each individual experiment included the concentration range of each peptide. The mixture was incubated for 5 min at 37°C and the reaction was interrupted by placing the samples in ice. They were then centrifuged at 600 *g* for 5 min at 4°C. Aliquots (0.1 ml) of the supernatant were taken for direct fluorometric assay of histamine (Håkanson & Rönnberg, 1974). The sediment was suspended in 1 ml boiling redistilled water and centrifuged at 600 *g* for 5 min at 4°C. Aliquots (0.1 ml) of the supernatant were assayed for histamine. All determinations were made in duplicate. The amount of histamine released was expressed as a percentage of the total amount of histamine present in the peritoneal cells at the start of the experiment. The results were corrected for 4–5% spontaneous release.

The pEC_{15} values (the negative logarithm of the concentration that releases 15% of the total histamine content) were estimated by interpolation from the pseudo-rectilinear portions of the concentration-response curves. Results are expressed as means ± s.e.mean; *n*, is the number of independent experiments. Statistical analysis of the difference between pEC_{15} values were performed by means of two-tailed unpaired Student's *t* test and modified by the Bonferroni method for multiple comparisons (Wallenstein *et al.*, 1980). $P < 0.05/4 = 0.012$ was considered significant.

Drugs

Porcine (p) NPY, NPY 22-36, human desamido-NPY (NPY without the C-terminal amide), pPYY and rat pancreatic polypeptide (PP) were purchased from Peninsula, UK. pNPY 15-36 and pPYY 15-36 were synthesized by solid-phase synthesis and purified to at least 96% by high performance liquid

[1] Author for correspondence.

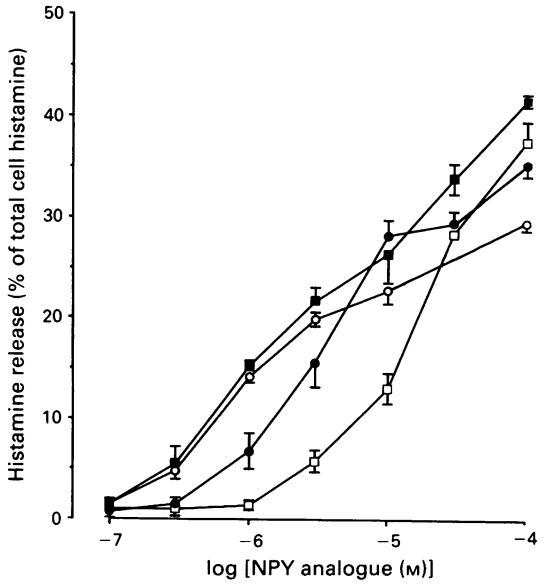

Figure 1 Concentration-response curves for histamine release from rat peritoneal mast cells evoked by neuropeptide (NPY) 1-36 (●), NPY 15-36 (○), NPY 22-36 (■) and NPY 26-36 (□). $n = 6$–9 independent experiments.

chromatography (h.p.l.c.) (W. Krzeminski, Ferring AB, Malmö, Sweden). NPY 26-36 was synthesized by solid phase synthesis and purified to at least 96% by h.p.l.c. (Dr H. Franzén, Dept. of Medical Chemistry, University of Lund, Sweden). p[Pro^{34}]NPY was a kind gift from Prof. T. W. Schwartz, Copenhagen, Denmark. Stock solutions were prepared and diluted with 0.9% saline.

Results

NPY, NPY 15-36, NPY 22-36, NPY 26-36 as well as desamido-NPY released histamine concentration-dependently from rat peritoneal mast cells (Figures 1 and 2). No concentration-response curve reached maximum. The pEC_{15} values for NPY 15-36 and NPY 22-36 were higher, while the pEC_{15} value for NPY 26-36 was lower than that for NPY (Table 1). At the highest concentration tested (0.1 mM) NPY, NPY 15-36, NPY 22-36 and NPY 26-36 released 30–40% of the total histamine content. The histamine release evoked by desamido-NPY was about 20% (Figure 2). In a series of experiments it was shown that the NPY-evoked histamine release reached maximum within 10 s (Figure 3).

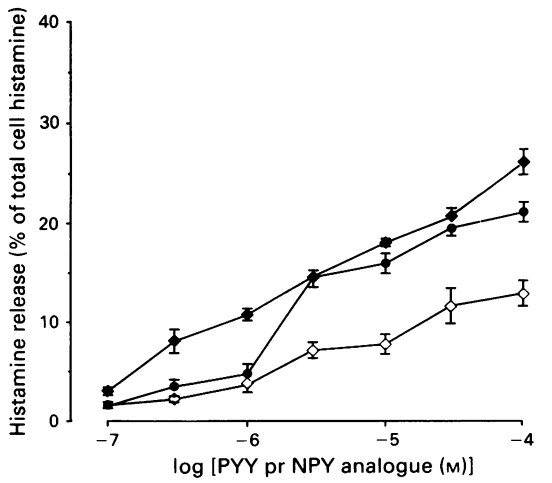

Figure 2 Concentration-response curves for histamine release from rat peritoneal mast cells evoked by peptide YY (PYY) 1-36 (◆), PYY 15-36 (◇) and desamido-NPY (●). $n = 3$–6 independent experiments.

Table 1 Histamine-releasing effect of neuropeptide Y (NPY), peptide YY (PYY), their C-terminal fragments and related peptides

	pEC_{15} values	n
NPY 1-36	5.6 ± 0.1	9
NPY 15-36	5.9 ± 0.0*	6
NPY 22-36	6.0 ± 0.1*	6
NPY 26-36	5.1 ± 0.1*	6
desamido-NPY	(+)	6
PYY	5.5 ± 0.1	5
PYY 15-36	(+)	3
[Pro^{34}]NPY	(+)	3
PP	(+)	3

Values are means ± s.e.mean from 3–6 independent experiments. * $P < 0.012$ for the difference between pEC_{15} values for analogues and fragments versus NPY 1-36. (+), histamine release less than 25% of the total histamine content at 0.1 mM peptide.

PYY and PYY 15-36 also released histamine concentration-dependently (Figure 2). The pEC_{15} values for PYY and NPY were similar (Table 1). PYY 15-36 was much less effective than PYY, since at 0.1 mM PYY released about 27%, while PYY 15-36 released about 13% of the total histamine content (Figure 2). [Pro^{34}]NPY and PP had virtually no effect. At the highest concentration tested (0.1 mM), [Pro^{34}]NPY and PP evoked a histamine release of 6.5 ± 0.3% ($n = 3$) and 7.2 ± 0.2% ($n = 3$), respectively.

Discussion

The depressor component of the biphasic blood pressure response to NPY in the anaesthetized rat can be abolished by pretreatment with histamine H_1-receptor antagonists or with the histamine liberator compound 48/80, implying that NPY is able to release histamine from a mast cell pool (Grundemar *et al.*, 1990). Similarly, several studies have shown that injections of short C-terminal NPY fragments evoke hypotension in the rat (Boublik *et al.*, 1989a, b; Scott *et al.*, 1990) and this response is also blocked by pretreatment with histamine H_1-receptor antagonists (Wahlestedt *et al.*, 1990).

The present study has demonstrated that NPY, NPY 15-36, NPY 22-36, NPY 26-36 as well as desamido-NPY concentration-dependently released histamine from rat peritoneal mast cells. Thus, the present results are in accordance with the suggestion that the hypotensive effect of NPY and its C-terminal fragments can be attributed to mast cell-dependent

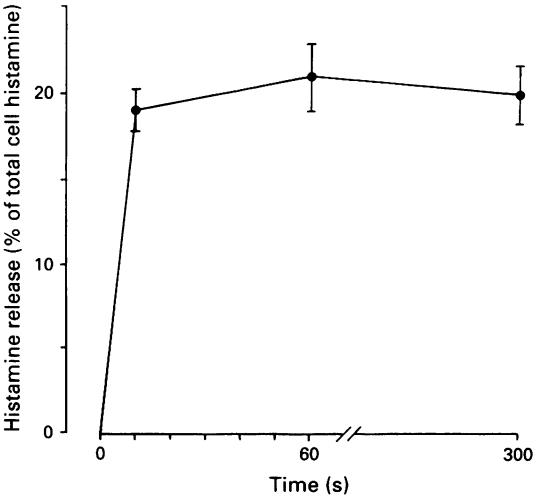

Figure 3 Time course of the neuropeptide Y (NPY)-evoked histamine release. In separate experiments NPY (10 μM, $n = 3$) was incubated for 10 s, 1 and 5 min, respectively.

release of histamine (Grundemar *et al.*, 1990; Wahlestedt *et al.*, 1990). Also PYY and PYY 15-36 evoked a concentration-dependent release of histamine, while PP and [Pro^{34}]NPY were virtually inactive.

NPY and PYY are thought to act on at least two receptor subtypes, referred to as Y_1 and Y_2 (Wahlestedt *et al.*, 1986, 1990). The Y_1-receptor seems to require the whole NPY/PYY molecule to become fully activated; whereas C-terminal fragments of the two peptides are markedly less potent (Rioux *et al.*, 1986; Wahlestedt *et al.*, 1986; Grundemar *et al.*, 1991). Recently, a selective Y_1-receptor agonist was constructed, [Pro^{34}]NPY, which seems to be equipotent with NPY on the Y_1-receptor, while having virtually no affinity to the Y_2-receptor (Krstenansky *et al.*, 1990), The Y_2-receptor recognizes not only the whole NPY/PYY molecule, but also C-terminal fragments of the two peptides. In fact, NPY 13-36/PYY 13-36 are only about one order of magnitude less potent than NPY/PYY (Wahlestedt *et al.*, 1986; Grundemar & Håkanson, 1990). Very short fragments, such as NPY 26-36/PYY 26-36 are inactive (Grundemar & Håkanson, 1990). PYY and NPY are approximately equipotent, while desamido-NPY exerts no activity on either receptor subtype (Wahlestedt *et al.*, 1986; Grundemar & Håkanson, 1990). PP displays 50% homology with NPY but has no activity on either of the Y_1-/Y_2-receptors.

The question arose as to how NPY activates mast cells to release histamine. Since NPY 15-36 and NPY 22-36 were at least as active as NPY and since the selective Y_1-receptor agonist [Pro^{34}]NPY was virtually inactive, the mast cells do not seem to express Y_1 receptors. PYY 15-36 was much less effective than either NPY or PYY, while NPY 26-36 was as effective as NPY but with a lower pEC_{15} value; these results are not in agreement with the properties of the Y_2-receptor. Moreover, desamido-NPY was active, albeit less so than the parent molecule. Taken together, the effect profile of the NPY/PYY-related peptides suggests that they release mast cell histamine by a mechanism distinct from the proposed Y_1-/Y_2-receptor subtypes. Conceivably, the positively charged amino acids of the NPY/PYY molecules activate G proteins in the mast cell membrane by a non-receptor mechanism, as has been suggested for tachykinins and other basic peptides (Mousli *et al.*, 1990). In support for a non-receptor mechanism, the kinetics of the histamine release by substance P and mastoparan are very rapid (less than 10 s) (Mousli *et al.*, 1989; 1990). Similarly, the NPY-evoked histamine release reached maximum within 10 s. Since PP and [Pro^{34}]NPY were inactive, the naturally occurring Pro^{34} in PP and the substitution of Pro^{34} in NPY could have an important conformational effect on the peptides, which may be sufficient to alter the relative disposition of the flanking arginine residues.

Whether the NPY-evoked release of mast cell histamine is physiologically relevant is unclear. Interestingly, mast cells are numerous around blood vessels and nerves (Stead *et al.*, 1989; Nilsson *et al.*, 1990). Possibly, the NPY-evoked release of mast cell histamine contributes to the sympathetic control in certain vascular beds.

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